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Original article

Synthesis, trypanocidal activity and molecular modeling studies of 2-alkylaminomethylquinoline derivatives

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ABSTRACT

Research and development of new drugs effective in the treatment of *Trypanosoma cruzi* infections are a real need for the 16 million people infected in the Americas. In a previous work, a quinoline derivative substituted by a 2-piperidylmethyl moiety showed to be active against Chagas disease and was considered a lead compound for further optimization. A series of ten analogous derivatives were tested against epimastigotes as a first approach. In view of their promising results, six of them were evaluated against the blood and intracellular replicative forms of the parasite in humans. Among them, compound **12** which possesses a 6-acetamidohexylamino substituent showed remarkable improvement in activity against epimastigotes, trypomastigotes and amastigotes compared with the structure lead, as well as a good selectivity index for the two parasite stages present in humans. In addition, treatment of infected mice with compound **12** induced a significant reduction in parasitemia compared with non-treated mice. Molecular modeling studies were performed by computational methods in order to elucidate the factors determining these experimental bioactivities.

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1. Introduction

Chagas disease, also known as American trypanosomiasis, is caused by the flagellate protozoan *Trypanosoma cruzi*. One century after its discovery, Chagas disease still remains a major health problem in Latin America [1]. In the past two decades, migration of thousands of paucisymptomatic infected persons from rural to urban areas in South America and from endemic regions to developed countries has changed the epidemiology of the disease [2,3]. Nowadays, it is an important public health issue in South America and an emerging disease in Europe and North America [4,5]. Chagas disease is characterized by an acute phase with high parasitemia, followed by an indeterminate stage that can last for years without signs or symptoms. Major complications, such as disability due to chronic cardiomyopathy and stroke, occur in 20–30% of patients in the chronic phase of disease [6].

Prophylactic and therapeutic vaccines have been pursued for decades but sterilizing immunity has not been achieved yet [7–10]. Therefore, chemotherapy remains the only treatment option for controlling infection once acquired, but none of the different chemotherapeutic strategies used in the past has proven consistently successful. Although other chemicals have been recently analyzed in the therapy of trypanosomiasis and several natural products such as quinone and terpene derivatives have also shown trypanocidal activity [11–13], the treatment modalities for trypanosomatid infections mostly rely on drugs that date back over 50 years and suffer from poor efficacy, high toxicity, and increasing resistance [14]. Accordingly, research and development of new drugs effective in the treatment of *T. cruzi* infections are a real need and novel strategies for drug design are required [15,16].

To develop other alternative drugs, new compounds have been searched based on empirical screening or ethnopharmacological studies. Thus, 2-substituted quinolines isolated from a Bolivian

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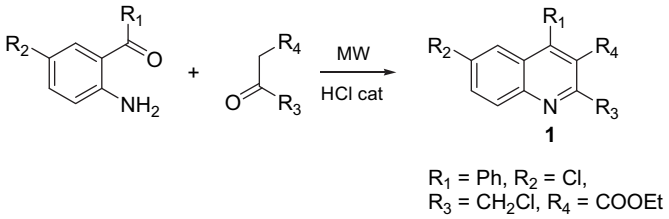
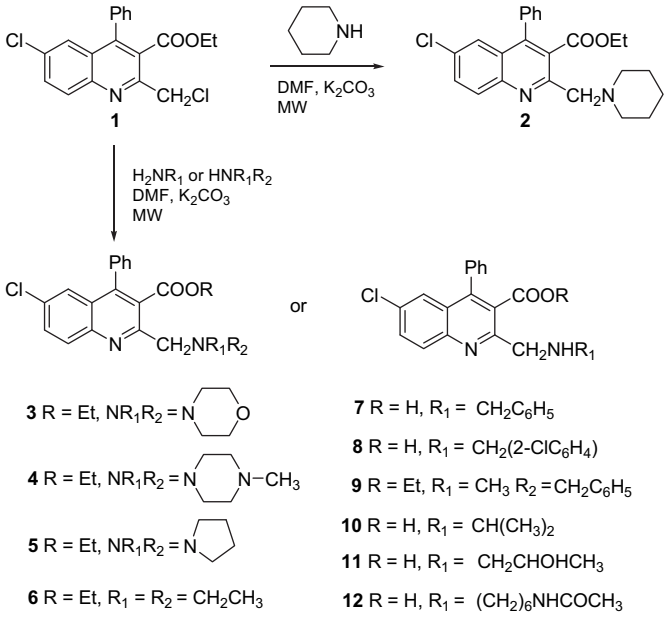


Scheme 1. Synthesis of quinolines *via* the Friedländer reaction.

medicinal plant, *Galipea longiflora* Kr (Rutaceae), have shown efficacy in the experimental treatment of cutaneous leishmaniasis as well as in Balb/c mice chronically infected with *T. cruzi* [17]. Later on, the synthesis of 2-substituted quinolines and their *in vitro* biological evaluation against the causal agent of Chagas disease among other parasites was reported [18]. Another series of quinolines were also synthesized and tested and one of them exhibited promising anti-trypanosomal activity [19]. Recently, a quinoline derivative having anticancer activity (tipifarnib) and synthesized analogs have shown strong activity against *T. cruzi* [20–22]. We have previously reported a series of polysubstituted quinolines synthesized *via* the Friedländer reaction employing microwave irradiation (MW) and a catalytic amount of hydrochloric acid (Scheme 1) [23]. These products were obtained in good yields at short times and were tested *in vitro* against the parasites causing malaria, leishmaniasis, sleeping sickness and Chagas disease at the Tropical Disease Research Laboratory, World Health Organization (WHO). This kind of structures had been designed considering the antiparasitic activity of 2-substituted quinoline alkaloids, many of which were isolated from medicinal plants [24,25].

Moreover, the 2-piperidylmethyl derivative **2** was prepared in good yields from the corresponding 2-chloromethylquinoline **1** under MW (Scheme 2) and was achieved in only 6 min instead of 3 h with conventional heating. This compound was moderately active against *Plasmodium falciparum* and active against *T. cruzi* epimastigotes [23]. Then, it was selected for a secondary *in vivo* screening at WHO and this assay was later repeated at higher doses (results not shown). In view of its remarkable performance,



Scheme 2. Synthesis of compound **2** and derivatives **3**–**12** (see Table 1)

compound **2** was considered a new lead compound for further optimization of trypanocidal activity.

In this work, the preparation of 2-alkylaminomethylquinoline-3-carboxylic acid derivatives (**3**–**12**) in good yields is described (Scheme 2 and Table 1). These syntheses were carried out in refluxing methylene chloride for conventional heating whereas under MW irradiation the reactions were neat.

The final products were evaluated *in vitro* against different developmental stages of *T. cruzi* (epimastigotes, trypomastigotes and amastigotes). With the aim of establishing preliminary structure–activity relationships (SAR) studies for these compounds, density functional theory (DFT) methods were employed to evaluate electronic properties such as molecular electrostatic potentials (MEPs), charge densities, dipole moments, and HOMO–LUMO energy values.

2. Results and discussion

2.1. Synthesis and anti-epimastigote activity

A series of ten 2-alkylaminomethylquinoline derivatives of the structure lead **2** (Table 1) were designed and synthesized by taking into account principles of medicinal chemistry such as isosterism, ring transformations, open-chain analogs and the inclusion of a suitable side chain containing a second protected amino group (compound **12**). This moiety was chosen because it is a recognized structure feature necessary for antimalarial activity of quinoline derivatives [26] and it is known that many compounds are active against both parasites. In addition, synthetic derivatives of the natural alkaloid piperine possessing different alkyl chain length with an amido group at the end, showed trypanocidal activity [27]. The acetamido group was selected just to protect the primary amine and could be assumed that it cleaves under test conditions to give the more reactive free primary amine.

The series of 2-alkylaminomethylquinoline derivatives were obtained by the nucleophilic substitution reaction of a variety of alkylamines and 2-chloromethyl-quinoline **1** under MW irradiation or conventional heating. Although the product yields were quite similar for both reaction-promoting methods, the reactions proceeded to completion in between 2 and 15 min when they were MW-assisted whereas otherwise 3–6 h were needed.

As first screening, the starting material **1**, the lead compound **2** and its ten analogs **3**–**12** were assayed *in vitro* against *T. cruzi* epimastigotes, using benznidazole as reference drug. Compounds **4**, **5**, **6** and **9** exhibited IC_{50} values against epimastigotes quite close to the lead compound (8.2 μM), whereas only compound **12**, which possesses a 6-acetamidohexylamino substituent, showed a remarkable improvement in activity (3.4 μM, Table 1). The replacement of the piperidyl moiety by N-methylpiperazinyl (**4**) caused a slight decrease in activity (IC_{50} 11.8 μM), as well as the ring contraction in the pyrrolidinyl derivative **5** (IC_{50} 10.0 μM) and its open-chain analog **6** (IC_{50} 11.6 μM). When the group benzylmethylamine was introduced (**9**), the resulting activity was also slightly decreased (IC_{50} 9.7 μM). The isosteric replacement of the piperidyl ring by morpholinyl (**3**) markedly decreased the activity (IC_{50} 85.3 μM). The introduction of benzyl and 2-chlorobenzyl moieties (**7** and **8**) led to products which could not be tested because of their low solubility. The presence of the *iso*-propyl group (**10**) as well as the 2-hydroxypropyl chain (**11**) decreased the activity against epimastigotes (IC_{50} 89.6 and 50.4 μM, respectively). It is worthy to note that the ethyl ester and the carboxylic acid groups attached at position 3 do not change the electronic properties of the whole quinoline nucleus and they do not influence the parameters for the nitrogen atom at position 2, according to our computational studies in this work and trypanocidal activity in previous results

Table 1
In vitro anti-trypanosomal and cytotoxic activity of compounds **1**–**12**.

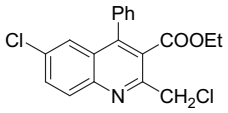
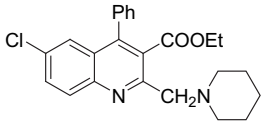
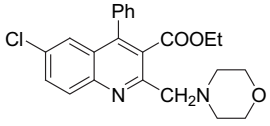
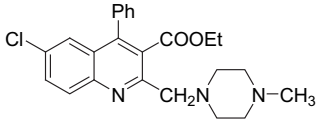
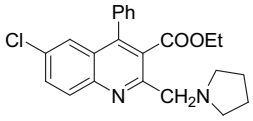
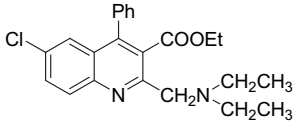
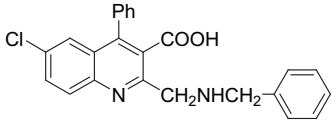
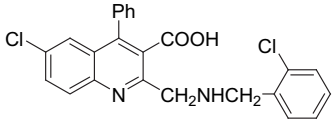
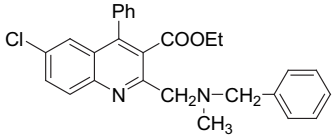
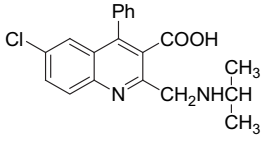
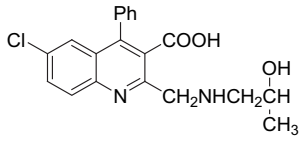
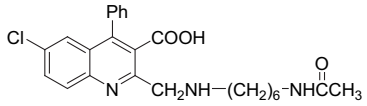
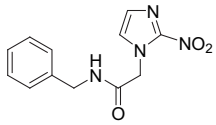
Compd	Structure	IC_{50} (μM) Epi[a]	IC_{50} (μM) Trypo[b]	IC_{50} (μM) Amas[c]	CC_{50} (μM)	SI Trypo	SI Amas
1		31.4	nd	nd	nd	nd	nd
2		8.2	51.3	37.9	310.6	6.05	8.2
3		85.3	nd	nd	nd	nd	nd
4		11.8	6.4	10.4	35.9	5.6	3.5
5		10.0	62.4	40.3	490.0	7.8	12.1
6		11.6	42.6	33.0	479.9	11.3	14.5
7		nd	nd	nd	nd	nd	nd
8		nd	nd	nd	nd	nd	nd
9		9.7	127.5	61.0	315.4	2.5	5.2
10		89.6	nd	nd	nd	nd	nd

Table 1 (*continued*)

Compd	Structure	IC_{50} (μM) Epi[a]	IC_{50} (μM) Trypo[b]	IC_{50} (μM) Amas[c]	CC_{50} (μM)	SI Trypo	SI Amas
11		50.4	nd	nd	nd	nd	nd
12		3.4	3.1	12.8	770.9	248.7	60.2
Bz[d]		5.8	34.1	3.6	706.0	20.70	196.1

nd: not determined.
[a] Epi: epimastigotes.
[b] Trypo: trypomastigotes.
[c] Amas: amastigotes.
[d] Bz: benznidazole.

[23]. Moreover, both groups can be found in active natural compounds [24].

2.2. In vitro activity against the blood and intracellular replicative forms

The promising finding of the antiparasitic activity of compounds **2**, **4**, **5**, **6**, **9** and **12** against *T. cruzi* epimastigotes prompted us to analyze their activity against the main stages found in humans: bloodstream trypomastigotes and the intracellular replicative amastigotes. We did not observe any significant differences between the activity against trypomastigotes and amastigotes when the piperidyl ring **2** (IC_{50} 51.3 and 37.9 μM, respectively) was replaced by the pyrrolidinyl ring **5** (IC_{50} 62.4 and 40.3 μM), or by its open-chain analog **6** (IC_{50} 42.6 and 33.0 μM). By contrast, the structural modification resulting from the introduction of N-methyl-N-benzyl **9** was not successful as an active compound against *T. cruzi* (IC_{50} 127.5 and 61.0 μM).

The substitution of piperidyl (**2**) by piperazinyl group (**4**) resulted in an incremental activity against trypomastigotes and amastigotes (IC_{50} 6.4 and 10.4 μM, respectively) compared to the lead compound (51.3 and 37.9 μM). It is possible that the N-methylpiperazinyl group could make compound **4** to reach easily the site of action of trypomastigotes and amastigotes compared with **2** because of the additional basic nitrogen atom and also its larger size owing to the methyl group. However, when the cytotoxic activity against the COS-7 cell line was analyzed, compound **4** was found to be toxic, having a CC_{50} value of 35.9 μM. A second amino group seems to be very important for the differential effect on amastigotes and trypomastigotes compared with epimastigotes, since compound **12** behaves similarly to compound **4** in this respect. Thus, an important decrease in the IC_{50} value was observed for compound **12** against trypomastigotes (3.1 μM) and amastigotes (12.8 μM). More importantly, the selectivity index (SI) used to compare the toxicity for mammalian cells and the activity against the parasites for compound **4** was 5.6 against trypomastigotes and 3.5 against amastigotes, while for the most active compound **12** it was 248.7 and 60.2, respectively, showing good selectivity for both parasite stages present in humans. This is particularly significant since a SI > 50 is considered adequate for trypanocidal drugs under development [28].

2.3. In vivo assay

In view of the promising results obtained *in vitro* with compound **12**, we analyzed the *in vivo* effect by treating infected mice for 10 days starting in day 5 post-infection. Untreated mice infected with *T. cruzi* trypomastigotes displayed high levels of parasitemia compared with those treated with compound **12** or benznidazole (Fig. 1). Considering the parasitemia curve throughout the acute phase of infection, calculated as the area under the parasitemia curve (AUC) [7], compound **12**-treated animals presented a threefold reduction in the number of parasites when compared to untreated ones (1.5 and 4.5×10^6, respectively). More importantly, at the peak of parasitemia (day 14), compound **12**-treated mice presented a significant reduction in parasitemia with respect to untreated ones ($6.2 \pm 1.6 \times 10^4$ versus $5.4 \pm 0.2 \times 10^5$ parasites/mL). Interestingly, the number of

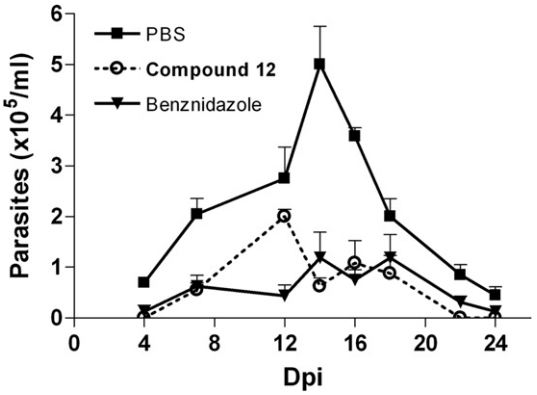


Fig. 1. Parasitemia levels during the acute infection period. C3H mice were infected with 5×10^3 *T. cruzi* bloodstream trypomastigotes and treated with compound 12, benznidazole or PBS. Data represent the mean ± SEM. Results presented are representative of three independent experiments.

circulating parasites of benznidazole-treated and compound **12**-treated animals was not significantly different (AUC: 1.3×10^6 and 1.5×10^6, respectively).

2.4. Molecular modeling studies

In order to elucidate the factors determining these experimental bioactivities, molecular modeling studies were performed by computational methods [29]. DFT geometry optimizations at the B3LYP/6-31G* level were carried out for all the structures. For the obtained potential energy minima, several physicochemical properties were analyzed in order to establish SAR: Mulliken charge densities, molecular electrostatic potentials (MEPs), dipole moments (μ), frontier molecular orbital energies (ε_{HOMO}, ε_{LUMO}), and chemical hardness ($\eta = (\varepsilon_{LUMO} - \varepsilon_{HOMO})$) [30,31]. Results are displayed in Table 2. HOMO and LUMO surfaces were also examined for derivative **12** (see supplementary material).

The molecular electrostatic potential (MEP) is the potential generated by the charge distribution of a molecule and denotes chemical reactivity, showing nucleophilic and electrophilic sites indicated by MEP contour maps [32]. Negative electrostatic potential corresponds to the attraction of a proton by the concentrated electron density in the molecule (lone pairs, pi-bonds); thus, revealing sites for electrophilic attack (colored in shades of red in standard contour diagrams). Positive electrostatic potential corresponds to the repulsion of a proton by the atomic nuclei in regions where low electron density exists and the nuclear charge is incompletely shielded (colored in shades of blue in standard contour diagrams).

The values in Table 2 did not reveal any correlation between the computed parameters and the assessed bioactivity. Neither did molecular orbital surfaces provide any further indication, as the surface shapes of the frontier orbitals were very similar in all the compounds. However, inspection of the MEP diagrams (see supplementary material) led to a plausible clarification of the activity data. All the studied compounds only differ in the substituent attached to the nitrogen atom of the 2-alkylaminomethyl group. According to the electrostatic potential maps, these substituents differently modify the degree of exposure of the nitrogen nonbonding electron pair and, consequently affect the accessibility to the electron density at this point. Therefore, this nitrogen atom was proposed as the reactive center for the studied compounds. In this way, the HOMO would be a proper descriptor of the activity. Nevertheless, the coefficient of the nitrogen considered in this molecular orbital was comparable for almost every derivative (Table 2). These observations suggest that relative activities of these series of compounds are not determined by electronic factors. Instead, the determined IC_{50} values could be governed by steric effects.

The structures of the active compounds illustrate the degree of steric hindrance exerted by the different substituents on the nitrogen atom of the 2-alkylaminomethyl group in common. The reduction in activity of derivative **6**, brought about by the highly rotational nature of its open-chain substituent should be noticed.

The lack of activity shown by derivative **3** could be attributed to the electron density of the oxygen atom in the morpholinyl substituent, being more available for electrophilic attack than the nitrogen atom mentioned above. This assumption would also apply to the hydroxyl group in compound **11**. This would also be the case for N-methylpiperazinyl derivative **4**, which showed a decrease in activity in relation to piperidyl analog **2**. The HOMO coefficient for the N atom under consideration was actually the lowest of the series (0.236), while the corresponding coefficient for the N-methyl atom was 0.293. On the other hand, the carbonyl group in the most active compound **12** is very distant from the proposed reactive nitrogen to influence the activity.

3. Conclusions

We herein show the syntheses of new 2-alkylaminomethylquino-line derivatives which exhibited a more selective anti-trypanosomal activity than the original lead compound (**2**). They could be obtained in good yields in a two-pathway simple reaction with affordable starting materials. The molecular modeling studies propose that the determined IC_{50} values could be governed by steric effects. Consequently, the trypanocidal activity could be improved if the nitrogen atom is linked to appropriate chains, allowing its nonbonding electron pair to be exposed.

Activity experiments showed that compound **12** is not only active against epimastigotes but also against amastigote and trypomastigote forms, which are the mammal stages of the parasite. Compound **12** differentially affects the parasite and the mammalian cell, with a significant selectivity index and is able to reduce parasitemia in infected mice. These characteristics make compound **12** an excellent candidate for extensive studies of *in vivo* activity and toxicity in murine models of acute and chronic *T. cruzi* infection.

4. Experimental methods

4.1. Chemistry

The structures of the synthesized compounds were established through their ^1H and ^{13}C NMR, MS and IR spectra. Melting points

Table 2
Bioactivities and computed molecular parameters for the compounds.

Compd	IC_{50} (μM)	μ (Debye)	q_N[a]	MEP_N (a.u.)[b]	HOMO (a.u.)	N_{HOMO}[c]	LUMO (a.u.)	η (a.u.)
12	3.4	4.1734	−0.565	−0.053	−0.2031	0.528	−0.0664	0.1368
2	8.2	2.7074	−0.407	−0.008	−0.2170	0.496	−0.0616	0.1554
9	9.7	3.4927	−0.363	−0.063	−0.1992	0.491	−0.0668	0.1324
5	10.0	3.7357	−0.367	−0.067	−0.2026	0.532	−0.0660	0.1366
6	11.6	2.3105	−0.351	−0.056	−0.1887	0.513	−0.0652	0.1235
4	11.8	3.5735	−0.418	−0.036	−0.1820	0.236	−0.0662	0.1158
1	31.4	3.1998	nd	nd	−0.2411	nd	−0.0742	0.1669
11	50.4	3.0390	−0.557	−0.053	−0.2053	0.515	−0.0643	0.1410
3	85.3	4.3046	−0.392	−0.032	−0.1986	0.464	−0.0670	0.1316
10	89.6	3.5826	−0.566	−0.054	−0.2136	0.544	−0.0652	0.1484
7	nd	3.2841	−0.548	−0.053	−0.2183	0.518	−0.0725	0.1458
8	nd	3.1617	−0.548	−0.051	−0.2146	0.522	−0.0691	0.1456

nd: not determined.
[a] Mulliken charge density at the nitrogen atom of interest (see text).
[b] MEP value at the nitrogen atom of interest (see text).
[c] HOMO coefficient for the nitrogen atom of interest (see text).

were determined in a capillary Electrothermal 9100 SERIES-Digital apparatus and are given uncorrected. ^{1}H and ^{13}C-NMR spectra were recorded at rt using a Bruker 300 MHz spectrometer. The operating frequency for protons and carbons was 300.13 and 75.46 MHz, respectively. The chemical shifts (δ) are given in ppm. IR spectra were recorded on a FT Perkin Elmer Spectrum One from KBr discs. Elemental analysis (C, H and N) was performed on an Exeter CE 440 and the results were within ±0.4% of the calculated values. Analytical TLCs were performed on DC-Alufolien Kieselgel 60 F_{254} Merck. Microwave-assisted reactions were carried out in a CEM Discover oven. All the alkyl (or heterocyclic) amines were commercially purchased, except N-(6-aminohexyl) acetamide which was prepared from a mixture of 1,6-diaminohexane (0.16 mol) and AcOEt (0.07 mol) stirred at rt for a week. The product was purified by fractionating distillation yield 76%; b.p. 162–164 °C (3 mmHg); ^{1}H NMR ($CDCl_3$): δ 1.27–1.32 (m, 4H), 1.39–1.49 (m, 4H), 1.66 (br, 1H), 1.93 (s, 3H), 2.62–2.67 (m, 3H), 3.16–3.22 (m, 2H), 5.90 (br,1H); ^{13}C NMR ($CDCl_3$): δ 23.19, 26.60, 29.44, 33.39, 39.44, 41.90, 42.00, 170.11; IR (cm^{-1}): υ 3418, 3067, 2927, 2848, 1646, 1377, 1300.

4.1.1. General procedure for compounds **3**–**12**

A mixture of **1** (0.40 g, 1.10 mmol), alkyl (or heterocyclic) amine (1.50 mmol), anhydrous K_2CO_3 (0.15 g, 1.10 mmol) was subjected to microwave irradiation, at 400 W. In the case of solid alkylamines, 0.3 mL DMF was added. After reaction completion (TLC), the reaction mixture was diluted with CH_2Cl_2 and washed with water, HCl 5% (10 mL) and brine, which was then dried (Na_2SO_4) and concentrated under reduced pressure to give a solid product which was triturated or crystallized from the proper solvent. Compound **10** could only be achieved under conventional heating, in refluxing CH_2Cl_2 as solvent, employing the same work-up.

4.1.2. Ethyl 6-chloro-2-(morpholin-4-ylmethyl)-4-phenylquinoline-3-carboxylate (**3**)

Yield 50%; mp 222–223 °C; reaction time 8 min; triturated with cyclohexane; ^{1}H NMR ($CDCl_3$): δ 0.82 (t, J = 7.2 Hz, 3H, CH_2CH_3), 3.59 (br, 4H, N–CH_2), 4.02 (q, J = 7.2 Hz 2H, CH_2CH_3), 4.08 (br, 4H, O–CH_2), 4.59 (s, 2H, Het-CH_2), 7.29–7.32 (m, 2H, Ph-H), 7.52 (m, 3H, Ph-H), 7.61 (d, J = 2.1 Hz, 1H, Het-H), 7.77 (dd, J = 9.0 Hz and 2.3 Hz, 1H, Het-H), 8.20 (d, J = 9.0 Hz, 1H, Het-H). ^{13}C NMR ($CDCl_3$): δ 13.33, 51.99, 58.25, 62.38, 63.91, 125.50, 126.90, 127.14, 128.63, 128.99, 129.18, 131.32, 132.54, 134.60, 134.77, 145.49, 146.16, 148.27, 166.86; IR (cm^{-1}): υ 3063, 1712, 1484, 1277, 835, 711, 610; Anal. Calcd. for $C_{23}H_{23}ClN_2O_3$: C, 67.23; H, 5.64; N, 6.82; found: C, 67.27; H, 5.59; N, 6.87.

4.1.3. Ethyl 6-chloro-2-(4-methylpiperazin-1-ylmethyl)-4-phenylquinoline-3-carboxylate (**4**)

Yield 68%; mp 237–239 °C; reaction time 5 min; crystallized from cyclohexane; ^{1}H NMR ($CDCl_3$): δ 0.93 (t, J = 7.1 Hz, 3H, CH_2CH_3), 2.74 (s, 3H, N–CH_3), 2.81–2.92 (m, 4H, N–CH_2), 3.08–3.12 (m, 2H, N–CH_2), 3.38–3.42 (m, 2H, N–CH_2), 4.01 (q, J = 7.1 Hz, 2H, CH_2CH_3), 4.09 (s, 2H, Het-CH_2), 7.31–7.34 (m, 2H, Ph-H), 7.51–7.53 (m, 3H, Ph-H), 7.57 (d, J = 2.1 Hz, 1H, Het-H), 7.69 (dd, J = 8.9 Hz and 2.3 Hz, 1H, Het-H), 8.07 (d, J = 9.1 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 13.68, 43.42, 49.01, 53.56, 61.13, 62.02, 125.27, 126.89, 127.14, 128.45, 128.85, 129.15, 131.07, 131.41, 133.31, 134.84, 145.59, 146.75, 154.52, 167.82; IR (cm^{-1}): υ 2980, 1719, 1604, 1226, 831, 761, 700; EI-MS m/z: 424 $[M + H]^+$; Anal. Calcd. for $C_{24}H_{26}ClN_3O_2$: C, 68.00; H, 6.18; N, 9.91; found: C, 68.05; H, 6.15; N, 9.87.

4.1.4. Ethyl 6-chloro-4-phenyl-2-(pyrrolidin-1-ylmethyl) quinoline-3-carboxylate (**5**)

Yield 70%; mp 199–201 °C, reaction time 2 min; crystallized from cyclohexane; ^{1}H NMR ($CDCl_3$): δ 0.82 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.16–2.30 (m, 4H, CH_2CH_2), 3.20–3.40 (br, 2H, N–CH_2), 3.98 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.15–4.40 (br, 2H, N–CH_2), 4.70 (s, 2H, Het-CH_2), 7.29–7.31 (m, 2H, Ph-H), 7.52–7.55 (m, 3H, Ph-H), 7.59 (d, J = 2.1 Hz, 1H, Het-H), 7.75 (dd, J = 9.1 Hz and 2.1 Hz, 1H, Het-H), 8.25 (d, J = 9.1 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 13.30, 24.10, 54.27, 56.20, 62.17, 125.45, 125.72, 126.83, 128.60, 128.97, 129.06, 131.50, 132.37, 134.42, 134.82, 145.55, 147.32, 148.14, 166.86; IR (cm^{-1}): υ 2997, 1718, 1619, 1221, 833, 703, 637; Anal. Calcd. for $C_{23}H_{23}ClN_2O_2$: C, 69.95; H, 5.87; N, 7.09; found: C, 69.91; H, 5.90; N, 7.12.

4.1.5. Ethyl 6-chloro-2-(diethylaminomethyl)-4-phenylquinoline-3-carboxylate (**6**)

Yield 65%; mp 177–179 °C; reaction time 14 min; crystallized from cyclohexane; ^{1}H NMR ($CDCl_3$): δ 0.90 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.56 (t, J = 7.0 Hz, 6H, N–CH_2CH_3), 3.55 (br, 2H, N–CH_2CH_3), 3.73 (br, 2H, N–CH_2CH_3), 4.07 (q, J = 7.1 Hz, 2H, CH_2CH_3), 4.63 (s, 2H, Het-CH_2), 7.32–7.34 (m, 2H, Ph-H), 7.53–7.55 (m, 3H, Ph-H), 7.62 (d, J = 2.2 Hz, 1H, Het-H), 7.77 (dd, J = 8.9 Hz and 2.1 Hz, 1H, Het-H), 8.20 (d, J = 8.9 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 9.98, 13.42, 49.03, 50.55, 62.44, 125.54, 126.58, 126.80, 128.67, 129.05, 129.24, 131.30, 132.41, 134.53, 134.55, 145.42, 147.24, 148.03, 166.71; IR (cm^{-1}): υ 2977, 1713, 1603, 1222, 838, 761, 708; Anal. Calcd. for $C_{23}H_{25}ClN_2O_2$: C, 69.60; H, 6.35; N, 7.06; found: C, 69.57; H, 6.31; N, 7.02.

4.1.6. 2-(Benzylaminomethyl)-6-chloro-4-phenylquinoline-3-carboxylic acid (**7**)

Yield 55%; mp 206–208 °C, reaction time 7 min; crystallized from EtOH; ^{1}H NMR ($CDCl_3$): δ 4.46 (s, 2H, NH–CH_2–Ph), 4.81 (s, 2H, Het-CH_2), 7.33–7.36 (m, 5H, Ph-H), 7.46–7.49 (m, 2H, Ph-H), 7.60–7.62 (m, 3H, Ph-H), 7.74 (dd, J = 9.0 Hz and 2.3 Hz, 1H, Het-H), 7.82 (d, J = 2.3 Hz, 1H, Het-H), 8.09 (d, J = 9.0 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 46.43, 50.30, 130.70, 126.24, 127.92, 128.04, 128.18, 128.45, 128.89, 129.17, 129.87, 130.69, 131.94, 132.87, 136.28, 147.08, 148.08, 160.81, 165.49; IR (cm^{-1}): υ 3436, 3067, 3033, 1698, 1613, 1227, 831, 758,700. Anal. Calcd. for $C_{24}H_{19}ClN_2O_2$: C, 71.55; H, 4.75; N, 6.95; found: C, 71.59; H, 4.71; N, 6.92.

4.1.7. 2-(2-Chlorobenzylaminomethyl)-6-chloro-4-phenylquinoline-3-carboxylic acid (**8**)

Yield 77%; mp 223–225 °C; reaction time 10 min; triturated with EtOH; ^{1}H NMR ($CDCl_3$): δ 4.56 (s, 2H, NH–CH_2–Ph), 4.98 (s, 2H, Het-CH_2), 7.24–7.61 (m, 9H, Ph-H), 7.76 (dd, J = 9.0 and 2.3 Hz, 1H, Het-H), 7.84 (d, J = 2.1 Hz, 1H, Het-H), 8.1 (d, J = 9.1 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 43.69, 50.77, 120.43, 126.24, 127.41, 128.02, 128.16, 129.17, 129.33, 129.72, 129.86, 130.64, 130.71, 131.93, 131.97, 132.89, 133.83, 133.91, 147.12, 148.12, 160.81, 165.62; IR (cm^{-1}): υ 3392, 3067, 1705, 1652, 1227, 832, 758, 702. Anal. Calcd. for $C_{24}H_{18}Cl_2N_2O_2$: C, 65.91; H, 4.15; N, 6.41; found: C, 65.94; H, 4.13; N, 6.44.

4.1.8. Ethyl 2-[(benzyl-methyl-amino)-methyl]-6-chloro-4-phenyl-quinoline-3-carboxylate (**9**)

Yield 77%; mp 142–145 °C; reaction time 10 min; triturated with EtOH; ^{1}H NMR ($CDCl_3$): δ 0.89 (t, J = 7.1 Hz, 3H, CH_2CH_3), 2.15 (s, 3H, N–CH_3), 3.55 (s, 2H, N–CH_2–Ph), 3.96 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.07 (s, 2H, Het-CH_2), 7.20–7.25 (m, 5H, Ph-H), 7.35–7.38 (m, 2H, Ph-H), 7.51–7.53 (m, 3H, Ph-H), 7.57 (d, J = 2.3 Hz, 1H, Het-H), 7.67 (dd, J = 8.8 Hz and 2.3 Hz, 1H, Het-H), 8.06 (d, J = 8.8 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 13.47, 41.78, 60.95, 61.66, 63.46, 125.35, 126.87, 127.02, 127.95, 128.33, 128.57, 129.11, 129.27, 130.87, 130.97, 132.76, 135.32, 138.38, 145.41, 146.25, 157.71, 167.86; IR (cm^{-1}): υ 2980, 1725, 1652, 1278, 836, 736, 703. Anal. Calcd. for $C_{27}H_{25}ClN_2O_2$: C, 72.88; H, 5.66; N, 6.30; found: C, 72.84; H, 5.68; N, 6.27.

4.1.9. 6-Chloro-2-(isopropylaminomethyl)-4-phenylquinoline-3-carboxylic acid (**10**)

Yield 70%; mp 230–232 °C; conventional heating 4 h; triturated with cyclohexane; ^{1}H NMR ($CDCl_3$): δ 1.31 (d, J = 6.9 Hz, 6H, CH_3), 4.53 (s, 2H, Het-CH_2), 4.61–4.72 (m, 1H, CH), 7.45–7.50 (m, 2H, Ph-H), 7.58–7.60 (m, 3H, Ph-H), 7.74–7.76 (m, 1H, Het-H), 7.77 (dd, J = 8.7 Hz and 2.3 Hz, 1H, Het-H), 8.12 (d, J = 8.7 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 20.48, 42.57, 45.85, 121.41, 126.20, 128.08, 129.02, 129.79, 130.56, 131.79, 132.10, 132.79, 146.75, 147.86, 161.01, 164.86; IR (cm^{-1}): υ 3043, 2970, 1689, 1607, 1225, 841, 763, 701. Anal. Calcd. for $C_{20}H_{19}ClN_2O_2$: C, 67.70; H, 5.40; N, 7.89; found: C, 67.75; H, 5.36; N, 7.87.

4.1.10. 2-[(2-Hydroxypropylamino)methyl]-6-chloro-4-phenylquinoline-3 carboxylic acid (**11**)

Yield 51%; mp 197–199 °C; reaction time 6 min; crystallized from EtOH; ^{1}H NMR ($CDCl_3$): δ 1.25 (d, J = 6.2 Hz, 3H, CH_3), 1.62 (br, 1H, OH), 2.53 (br, 1H, NH), 3.57 (dd, J = 14.4 Hz and 7.9 Hz, 1H, CH_2), 3.70 (dd, J = 14.4 Hz and 3.3 Hz, 1H, CH_2), 4.17 (m, 1H, CH), 4.69 (d, J = 17.4 Hz, 1H, Het-CH_2), 4.77 (d, J = 17.4 Hz, 1H, Het-CH_2), 7.39–7.43 (m, 2H, Ph-H), 7.54–7.58 (m, 3H, Ph-H), 7.74 (d, J = 2.4 Hz, 1H, Het-H), 7.78 (dd, J = 8.9 Hz and 2.4 Hz, 1H, Het-H), 8.10 (d, J = 9.1 Hz, 1H, Het-H); ^{13}C NMR ($CDCl_3$): δ 21.43, 50.76, 52.90, 67.37, 126.23, 128.11, 129.15, 129.76, 130.63, 132.01, 147.99, 160.99; IR (cm^{-1}): υ 3415, 2977, 1669, 1608, 1230, 838, 761, 702. Anal. Calcd. for $C_{20}H_{19}ClN_2O_3$: C, 64.78; H, 5.16; N, 7.55; found: C, 64.75; H, 5.19; N, 7.52.

4.1.11. 2-[6-(Acetamidohexylamino)methyl]-6-chloro-4-phenylquinoline-3-carboxylic acid (**12**)

Yield 56%; mp 136–139 °C; reaction time 7.5 min; triturated with cyclohexane; ^{1}H NMR ($CDCl_3$): δ 1.36–1.43 (m, 4H, CH_2), 1.59–1.69 (m, 5H, CH_2, NH), 1.80 (s, 3H, $COCH_3$), 3.08–3.14 (m, 2H, CH_2NHCO), 3.58 (t, J = 7.1 Hz, 2H, NHCH_2), 4.50 (s, 2H, Het-CH_2), 7.34–7.36 (m, 2H, Ph-H), 7.49–7.51 (m, 3H, Ph-H), 7.68–7.73 (m, 2H, d, Het-H), 8.06 (d, J = 8.9 Hz, 1H, Het-H); IR (cm^{-1}): υ 3343, 2929, 1689, 1641, 1239, 833, 758, 699. Anal. Calcd. for $C_{25}H_{28}ClN_3O_3$: C, 66.14; H, 6.22; N, 9.26; found: C, 66.17; H, 6.25; N, 9.24.

4.2. Computational procedures

DFT full geometry optimizations were performed with the Gaussian 03 program [29] using the B3LYP functional [33–35] and the 6-31G* basis set. Molecular parameters of the optimized minima were evaluated at the same level of theory. Distribution of the electrostatic potential derived from the electron density of the compounds was estimated by energy calculations at the optimized structures.

4.3. Biology

4.3.1. In vitro trypanocidal activity assay

T. cruzi epimastigotes (RA strain) were grown in biphasic medium and maintained by weekly passages at 26 °C. *T. cruzi* bloodstream trypomastigotes were obtained from infected CF1 mice by cardiac puncture at the peak of parasitemia on day 15 post-infection. Trypomastigotes were routinely maintained by infecting 21-day-old CF1 mice.

Growth inhibition of *T. cruzi* (RA) epimastigotes was evaluated by a [^{3}H] thymidine uptake assay as previously described in Ref. [11]. Compounds were tested at 100, 50, 10, 5, 1 and 0.5 μg/mL (final concentration). Briefly, parasites were adjusted to a cell density of 1.5 × 10^6/mL and cultured in the presence of compounds for 72 h. Benznidazole (5–20 μM; Roche) was used as positive control. The percentage of inhibition was calculated as 100 − {[(cpm of treated parasites)/(cpm of untreated parasites)] × 100}, and the IC_{50} was estimated.

The trypanocidal effect of the compound was also tested on bloodstream trypomastigotes as previously described in Ref. [12]. Briefly, mouse blood containing trypomastigotes was diluted in complete RPMI medium to adjust parasite concentration to 1.5 × 10^6/mL. Parasites were seeded (150 μL/well) in duplicate in a 96-well microplate and 2 μL of the compound/well (0.5–100 μg/mL final concentration), or control drug (benznidazole) were added. Plates were incubated for 24 h at 4 °C and the remaining live parasites were counted in a Neubauer chamber. The percentage of inhibition was calculated as 100 − {[(live parasites in wells after compound treatment)/(live parasites in untreated wells)] × 100}.

4.3.2. Cytotoxicity assay

African green monkey kidney (COS-7) cell lines were employed for the determination of cell viability by the MTT assay. Cells (6 × 10^4) were settled in a 96-well flat-bottom microtiter plate in the absence and presence of increasing concentrations of the compounds (10–500 μg/mL) and incubated at 37 °C 5% CO_2 for 24 h. For the last 4 h, 20 μL of MTT solution (1.5 mg/mL in complete RPMI medium without phenol red (Gibco) containing 10% fetal bovine serum) was added into each well. The purple formazan crystals were completely dissolved by adding 150 μL of ethanol and absorbance was detected at 570 nm in a microplate reader. The results were calculated as the relationship between viable cells in the presence and absence of the compound × 100. The selectivity index (SI) was calculated as the 50% cytotoxic concentration (CC_{50}) divided by the 50% inhibitory concentration (IC_{50}) of the compound for *T. cruzi* trypomastigotes and amastigotes.

4.3.3. Amastigote growth inhibition assay

Ninety-six well tissue culture plates (Sarstedt Inc., Newton, NC) were seeded with a murine macrophage cell line, J774, at 5 × 10^3 per well in 100 μL volumes and incubated 2 h at 37 °C 5% CO_2. Cells were infected with transfected trypomastigotes expressing β-galactosidase [36], at a parasite cell ratio 10:1, kindly provided by Dr. Buckner. After 24 h of co-culture, plates were washed twice with PBS to remove unbound parasites and drug compounds were added at 0.5–100 μg/mL per well in 200 μL fresh complete RPMI medium without phenol red. Each concentration was tested in duplicate. Controls included uninfected JTT4 cells with RPMI alone (0% infection control) and cell monolayers infected with trypomastigotes (100% infection control). On day 7, the assays were developed by addition of CPRG (100 μM final concentration) and Nonidet P-40 (1% final concentration). Plates were incubated for 4–6 h at 37 °C. Wells with galactosidase activity turned the media from yellow to red, and this was quantitated using a microplate reader (A570 nm, Bio-Rad Laboratories). The percentage of inhibition was calculated as 100 − {[(Absorbance of treated infected cells)/(Absorbance of untreated infected cells)] × 100}, and the IC_{50} was estimated.

4.3.4. Animals

Female C3H/HeN mice were nursed at the Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires. Mice were housed in groups of 5 per cage. They were kept in a conventional room at 24 ± 1 °C with free access to a standard commercial diet and water ad libitum under a 12 h light/12 h dark cycle. All procedures were approved by the Ethics Review Board of the Instituto de Estudios de la Inmunidad Humoral (IDEHU-CONICET) and conducted in accordance with the guidelines established by the National Research Council, Guide for the Care and Use of Laboratory Animals [37].

4.3.5. In vivo assays

Groups of five female C3H/HeN mice (6–8 week-old, 23.8 ± 2.6 g), were infected with 5 × 10^3 bloodstream *T. cruzi*

trypomastigotes (RA strain) by intraperitoneal injection [8,38]. Mice were daily treated with either 1 mg/kg of body weight/day of compound **12**, benznidazole or PBS (as control) for 10 consecutive days (days 5–15 post-infection). Parasitemia was individually monitored, after lysis of red cells, by direct microscopic of parasite count in 5 μL of blood employing a hemocytometer.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2011.05.035

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